

U.S.S.N. 08/259,321

Filed: June 10, 1994

AMENDMENT

a

*Sub
B
cont*

TGTTCTCTCT CTGGGTTTTC ACTGAGGACT TCTGGTATGG GTGTAGGCTG
GATTCGTCAG CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA CACATTTGGT
GGGATGATGA CAAGCGCTAT AACCCAGTCC TGAAGAGCCG ACTGATAATC
TCCAAGGATA CCTCCAGGAA ACAGGTATTC CTCAAGATCG CCAGTGTGGA
CACTGCAGAT ACTGCCACAT ACTACTGTGT TCGAATGATG GATGATTACG
ACGCTATGGA CTACTGGGGT CAAGGAACCT CAGTCACCGT CTCCTCT (Sequence
ID No. 9); CAG GTTACTCTGA AAGAGTCTGG CCCTGGGATA TTGCAGCCCT
CCCAGACCCT CACTCTGACT TGTTCTCTCT CTGGGTTTTC ACTGAGGACT
TCTGGTATGG GTGTAGGCTG GATTCGTCAG CCTTCAGGGA AGGGTCTGGA
GTGGCTGGCA CACATTTGGT GGGATGATGA CAAGCGCTAT AACCCAGTCC
TGAAGAGCCG ACTGATAATC TCCAAGGATA CCTCCAGGAA ACAGGTATTC
CTCAAGATCG CCAGTGTGGA CACTGCAGAT ACTGCCACAT ACTACTGTGT
TCGAATGATG GATGATTACG ACGCTATGGA CTACTGGGGT CAAGGAACCT
CAGTCACCGT CTCCTCT (nucleotides 58 to 417 of Sequence ID No. 9); ATGGATTTTC
AGGTGCAGAT TTTCAGCTTC CTGCTAATCA GTGCCTCAGT CATAATGTCC
AGAGGACAAA TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT
GGGGGAGGAG ATCACCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG
TCCACTGGTA CCAGCAGAAG TCAGGCACTT CTCCCAAACCT CTGATTTAT
GGGACATCCA ACCTGGCTTC TGGAGTCCCT TCTCGTTTCA GTGGCAGTGG
GTCTGGGACC TTTTATTCTC TCACAGTCAG CAGTGTGGAG GCTGAAGATG
CTGCCGATTA TTAAGTCCAT CAGTGGAATA GTTATCCGCA CACGTTCCGA

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Sub B cont
Q1
GGGGGGACCA AGCTGGAAAT AAAACGG (Sequence ID No. 11); CAAA
TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT GGGGGAGGAG
ATCACCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG TCCACTGGTA
CCAGCAGAAG TCAGGCACTT CTCCCAAACCT CTTGATTTAT GGGACATCCA
ACCTGGCTTC TGGAGTCCCT TCTCGTTTCA GTGGCAGTGG GTCTGGGACC
TTTTATTCTC TCACAGTCAG CAGTGTGGAG GCTGAAGATG CTGCCGATTA
TTACTGCCAT CAGTGGAATA GTTATCCGCA CACGTTCCGA GGGGGGACCA
AGCTGGAAAT AAAACGG (nucleotides 67 to 387 of Sequence ID No. 11); and degenerate
sequences thereof, and wherein the antibody is not the HPC-4 antibody deposited with the
American Type Culture Collection as ATCC No. HB 9892.

Sub F1
2. (amended) The antibody of claim 1 comprising an amino acid sequence selected from the group consisting of:

MGRLLLLL LIAPAYVLSQ VTLKESGPGI LQPSQTLTLT CSLSGFSLRT
SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII SKDTSRKQVF
LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Sequence ID No. 10);
MDFQVQIFSF LLISASVIMS RGQILTQSP AIMSASLGEE ITLTCSATSS
VTYVHWYQQK SGTSPKLLIY GTSNLAGVP SRFSGSGSGT FYSLTVSSVE
AEDAADYYCH QWNSYPHTFG GGTKLEIKR (Sequence ID No. 12); Q VTLKESGPGI
LQPSQTLTLT CSLSGFSLRT SGMGVGWIRQ PSGKGLEWLA HIWWDDDKRY
NPVLKSRLII SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG
QGTSVTVSS (amino acids 20-139 of Sequence ID No. 10) and QILTQSP AIMSASLGEE

mature seq - do minus leader

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Sub 1
cut
ITLTCSATSS VTYVHWYQQK SGTSPKLLIY GTSNLAGVVP SRFSGSGSGT
FYSLTVSSVE AEDAADYYCH QWNSYPHTFG GGTKLEIKR (amino acids 23-129 of
Sequence ID No. 12). *HPC-4 V4*
natural peptide

Sub B2
3. (amended) The antibody of claim 1 containing human amino acid sequence other than the sequence defining the epitope binding specificity.

Please cancel claim 4.

A2
5. (amended) [The] A composition comprising the antibody of claim 1 [further comprising] in combination with a pharmaceutically acceptable carrier for administration to a patient.

Please cancel claim 6.

A3
8. (amended) The antibody of claim 1 immobilized to a substrate which does not interfere with binding of the antibody to protein C in combination with calcium ions, wherein the immobilized antibody is suitable for purification of protein C from a biological fluid.

Please cancel claims 9-13.

A4 *Sub B3*
14. (amended) A method of making a recombinant Ca^{2+} dependent monoclonal antibody immunoreactive with [an] a first epitope in the activation peptide region of the heavy chain of Protein C defined by E D Q V D P R L I D G K (Sequence ID No. 1) in combination with a second epitope consisting of calcium ions, where the antibody inhibits Protein C activation by thrombin-thrombomodulin, by expressing nucleotide sequence encoding the antibody, wherein the antibody is encoded in part by a nucleotide sequence selected from the group consisting of ATGGGCAGGC TTTCTTCTTC ATTCTTGCTA

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Sub
B3
cont.

CTGATTGCCC CTGCATATGT CCTGTCCCAG GTTACTCTGA AAGAGTCTGG
CCCTGGGATA TTGCAGCCCT CCCAGACCCT CACTCTGACT TGTCTCTCT
CTGGGTTTTC ACTGAGGACT TCTGGTATGG GTGTAGGCTG GATTCGTCAG
CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA CACATTGGT GGGATGATGA
CAAGCGCTAT AACCCAGTCC TGAAGAGCCG ACTGATAATC TCCAAGGATA
CCTCCAGGAA ACAGGTATTC CTCAAGATCG CCAGTGTGGA CACTGCAGAT
ACTGCCACAT ACTACTGTGT TCGAATGATG GATGATTACG ACGCTATGGA
CTACTGGGGT CAAGGAACCT CAGTCACCGT CTCCTCT (Sequence ID No. 9); CAG
GTTACTCTGA AAGAGTCTGG CCCTGGGATA TTGCAGCCCT CCCAGACCCT
CACTCTGACT TGTCTCTCT CTGGGTTTTC ACTGAGGACT TCTGGTATGG
GTGTAGGCTG GATTCGTCAG CCTTCAGGGA AGGGTCTGGA GTGGCTGGCA
CACATTGGT GGGATGATGA CAAGCGCTAT AACCCAGTCC TGAAGAGCCG
ACTGATAATC TCCAAGGATA CCTCCAGGAA ACAGGTATTC CTCAAGATCG
CCAGTGTGGA CACTGCAGAT ACTGCCACAT ACTACTGTGT TCGAATGATG
GATGATTACG ACGCTATGGA CTACTGGGGT CAAGGAACCT CAGTCACCGT
CTCCTCT (nucleotides 58 to 417 of Sequence ID No. 9); ATGGATTTTC AGGTGCAGAT
TTTCAGCTTC CTGCTAATCA GTGCCTCAGT CATAATGTCC AGAGGACAAA
TTATTCTCAC CCAGTCTCCG GCAATCATGT CTGCATCTCT GGGGGAGGAG
ATCACCTAA CCTGCAGTGC CACTTCGAGT GTAACCTACG TCCACTGGTA
CCAGCAGAAG TCAGGCACTT CTCCCAAACCT CTTGATTAT GGGACATCCA
ACCTGGCTTC TGGAGTCCCT TCTCGTTTCA GTGGCAGTGG GTCTGGGACC

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Sub
B3
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TTTTATTCTC TCACAGTCAG CAGTGTGGAG GCTGAAGATG CTGCCGATTA
TTACTGCCAT CAGTGGAATA GTTATCCGCA CACGTTCGGA GGGGGGACCA
AGCTGGAAAT AAAACGG (Sequence ID No. 11); CAAA TTATTCTCAC
CCAGTCTCCG GCAATCATGT CTGCATCTCT GGGGGAGGAG ATCACCCTAA
CCTGCAGTGC CACTTCGAGT GTAACCTACG TCCACTGGTA CCAGCAGAAG
TCAGGCACTT CTCCCAAACCT CTTGATTAT GGGACATCCA ACCTGGCTTC
TGGAGTCCCT TCTCGTTTCA GTGGCAGTGG GTCTGGGACC TTTTATTCTC
TCACAGTCAG CAGTGTGGAG GCTGAAGATG CTGCCGATTA TTACTGCCAT
CAGTGGAATA GTTATCCGCA CACGTTCGGA GGGGGGACCA AGCTGGAAAT
AAAACGG (nucleotides 67 to 387 of Sequence ID No. 11); and degenerate sequences
thereof, and is not HPC-4 antibody as deposited with the American Type Culture Collection
as ATCC No. HB 9892.

15. (amended) The method of claim 14 wherein the antibody comprises an amino acid sequence selected from the group consisting of:

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MGR LSSS FLL LIAPAYVLSQ VTLKESGPGI LQPSQTLTLT CSLSGFSLRT
SGMGVGVWIRQ PSGKGLEWLA HIWWDDDKRY NPVLKSRLII SKDTSRKQVF
LKIASVDTAD TATYYCVRMM DDYDAMDYWG QGTSVTVSS (Sequence ID No. 10);
MDFQVQIFS L LISASVIMS RGQILTQSP AIMSASLGEE ITLTCSATSS
VTYVHWYQQK SGTSPKLLIY GTSNLASGVP SRFSGSGSGT FYSLTVSSVE
AEDAADYYCH QWNSYPHTFG GGTKLEIKR (Sequence ID No. 12); Q VTLKESGPGI
LQPSQTLTLT CSLSGFSLRT SGMGVGVWIRQ PSGKGLEWLA HIWWDDDKRY

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NPVLKSRLII SKDTSRKQVF LKIASVDTAD TATYYCVRMM DDYDAMDYWG

QGTSVTVSS (amino acids 20-139 of Sequence ID No. 10) and QIILTQSP AIMSASLGEE

ITLTCSATSS VTYVHWYQQK SGTSPKLLIY GTSNLAGGVP SRFSGSGSGT

FYSLTVSSVE AEDAADYYCH QWNSYPHTFG GGTKLEIKR (amino acids 23-129 of
Sequence ID No. 12).

Please cancel claim 16.

17. (amended) The method of claim 14 further comprising inserting human sequence into the antibody in place of animal sequence other than the sequence defining the epitope binding specificity.

19. (amended) The method of claim 14 further comprising immobilizing the antibody to a substrate which does not interfere with binding of the antibody to protein C in combination with calcium ions, wherein the immobilized antibody is suitable for purification of protein C from a biological fluid.

Please add the following new claims.

20. A recombinant HPC-4 antibody as deposited with the American Type Culture Collection as ATCC No. 9892 expressed as a fusion protein.

21. A method for making a recombinant HPC-4 antibody wherein a nucleotide sequence encoding HPC-4 antibody as deposited with the American Type Culture Collection as ATCC No. 9892 is ligated to a sequence encoding a different protein and expressed in an expression system as a fusion protein.